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Abnormal swimming behavior and increased deformities in rainbow trout *Oncorhynchus mykiss* cultured in low exchange water recirculating aquaculture systems

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ABSTRACT

Two studies were conducted to evaluate rainbow trout *Oncorhynchus mykiss* health and welfare within replicated water recirculating aquaculture systems (WRAS) that were operated at low and near-zero water exchange, with and without ozonation, and with relatively high feed loading rates. During the first study, rainbow trout cultured within WRAS operated with low water exchange (system hydraulic retention time (HRT) = 6.7 days; feed loading rate = 4.1 kg feed/m^3 daily makeup flow) exhibited increased swimming speeds as well as a greater incidence of "side swimming" behavior as compared to trout cultured in high exchange WRAS (HRT = 0.67 days; feed loading rate = 0.41 kg feed/m^3 daily makeup flow). During the second study, when the WRAS were operated at near-zero water exchange, an increased percentage of rainbow trout deformities, as well as increased mortality and a variety of unusual swimming behaviors were observed within WRAS with the highest feed loading rates and least water exchange (HRT ≥ 103 days; feed loading rate ≥ 71 kg feed/m^3 daily makeup flow). A wide range of water quality variables were measured. Although the causative agent could not be conclusively identified, several water quality parameters, including nitrate nitrogen and dissolved potassium, were identified as being potentially associated with the observed fish health problems.

1. Introduction

Water recirculating aquaculture systems (WRAS) offer many advantages (Summerfelt and Vinci, 2008); however, recent studies have indicated that accumulating water quality concentrations could be problematic when these systems are operated with minimal water exchange. Several studies have examined the effects of accumulating water quality parameters within low exchange WRAS on the performance of various species including: common carp (Martins et al., 2009a); hybrid striped bass *Morone chrysops* × *Morone saxatilis* and tilapia *Oreochromis* spp. (Brazil, 1996; Martins et al., 2009b); European sea bass *Dicentrarchus labrax* (Deviller et al., 2005), and rainbow trout *Oncorhynchus mykiss* (Davidson et al., 2009; Good et al., 2009). Martins et al. (2009a) concluded that ortho-phosphate-P, nitrate, and heavy metals (arsenic and copper) accumulated to levels that likely impaired the embryonic and larval development of common carp. Martins et al. (2009b) reported that larger tilapia showed a trend towards growth retardation in the lowest flushing WRAS, but small individuals seemed to grow faster in such systems. Deviller et al. (2005) attributed a 15% growth reduction in European sea bass cultured within WRAS to an unknown "growth-inhibiting substance" and suggested that metals accumulation could have contributed to reduced fish performance. Davidson et al. (2009) concluded that certain water quality constituents (e.g., dissolved copper, total suspended solids, and fine particulate matter) can accumulate to concentrations that are potentially harmful to salmonid performance and welfare when makeup water is reduced within WRAS and systems are operated with relatively high feed loading rates (≥4 kg daily feed per m^3 daily makeup water).

Other studies have also indicated that certain water quality constituents measured within fish culture systems can cause skeletal deformities. Baeverfjord et al. (2009a) reported that anecdotal evidence from intensive Atlantic salmon *Salmo salar* smolt production trials indicated that some aspect of the water quality was associated with skeletal deformity, but could not pinpoint a specific parameter. Additionally, Baeverfjord et al. (2009b) attributed increasing levels of carbon dioxide (up to 30 mg/L) to a shortening of the body in cultured rainbow trout. Shimura et al. (2004) suggested that elevated nitrate nitrogen (100 mg/L) contributed to skeletal deformity observed in juvenile Medaka *Oryzias latipes*

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during a long-term toxicity challenge in aquaria. Many studies have indicated that elevated concentrations of various water quality parameters in natural settings have caused increased skeletal deformities in fish including: heavy metals (Bengtsson et al., 1988; Lall and Lewis-McCrea, 2007); zinc (Bengtsson, 1974; Sun et al., 2009); cadmium (Pragatheeswaran et al., 1987), lead (Sun et al., 2009); selenium (Lemly, 2002); ammonium and low dissolved oxygen (Sun et al., 2009). Lall and Lewis-McCrea (2007) suggested that skeletal deformities in fish could also be caused by insecticides, pesticides, organochlorine, and other chemicals. Many of the aforementioned studies also discussed changes in fish behavior that were likely associated with elevated water quality concentrations.

A series of controlled studies have been conducted in six replicated WRAS to identify how fish growth, survival, health, and welfare metrics are impacted under various culture conditions (Davidson et al., 2009, 2011; Good et al., 2009, 2010). The primary objective of this paper is to describe fish health and welfare observations (unusual swimming behaviors, increased prevalence of deformities, and decreased survival) from several of these studies (Davidson et al., 2011), as well as the corresponding water quality conditions, when WRAS were operated at low and near-zero water exchange.

2. Methods

2.1. Experimental systems and treatments

Rainbow trout performance, health, and welfare metrics as well as system water quality were evaluated within six identical 9.5 m^3 WRAS during two studies. These systems are described in detail in Davidson et al. (2009, 2011). Treatment metrics for the present studies are outlined in Table 1. Study 1 – Three WRAS were operated with “low” water exchange and ozone vs. three WRAS operated with “high” water exchange without ozone. Mean system hydraulic retention times (HRT) for the low and high exchange WRAS were approximately 6.7 and 0.67 days, respectively; and mean feed loading rates were 4.1 and 0.41 kg feed per cubic meter of daily makeup water, respectively (Davidson et al., 2011). WRAS described as operating at low and high water flushing rates continuously exchanged 0.26% and 2.6% of the total recycled flow. Study 2 – The original study design was to evaluate three WRAS operated at near-zero water exchange (i.e., backwash replacement only) with ozone compared to three WRAS operated at near-zero water exchange without ozone. During this study, periodic drum filter failures occurred within four of six WRAS which resulted in increased and variable dilution amongst WRAS. Additionally, drum filter backwash spray was found to be added as additional makeup water to some WRAS and not others, which also contributed to differences in dilution. Due to the variability in flushing during Study 2, individual WRAS turnover rates varied from <10 days to as high as 180 days and feed loading rates ranged from 4 to 147 kg feed per cubic meter of daily makeup water. In order to evaluate the potential correlation of feed loading rate and accumulating water quality to the observed fish health and welfare issues during the present studies, WRAS were separated into two treatment groups based on feed loading rate and HRT: (1) very low exchange – WRAS with mean HRT's of $\leq$36 days and mean feed loading rates $\leq$44 kg feed/m^3 makeup water/day compared to (2) near-zero exchange – WRAS with HRT's $\geq$103 days and feed loading rates $\geq$71 kg feed/m^3 makeup water/day. For comparative purposes, data generated from WRAS 3 was excluded. WRAS 3 had the least flushing of any WRAS and also used ozone; therefore this system could not be categorized with other WRAS that did not use ozone and had significantly different flushing rates.

2.2. Rainbow trout

All female, diploid, rainbow trout (Kamloops strain) obtained as eyed eggs from Troutlodge Inc. (Sumner, WA, USA) were used. All experimental fish were hatched on-site within a recirculating incubation system and then cultured within flow through systems prior to use in the present studies. Equal numbers of fish were stocked in each WRAS to begin each study. Rainbow trout were 151 ± 3 g to begin Study 1 and 18 ± 1 g to begin Study 2. Initial stocking densities for Studies 1 and 2 were 30 and 12 kg/m^3, respectively. Maximum densities were maintained at $\leq$80 kg/m^3.

2.3. Photoperiod and feeding

A constant 24-h photoperiod was provided. Fish were fed a standard 42:16 trout diet (Zeigler Brothers, Inc., Gardners, PA, USA). Equal daily rations were delivered to each WRAS with feeding events occurring every other hour, around the clock, using automated feeders (T-drum 2000CE, Arvo-Tec, Finland). Additional detail relative to feeding methodology was described in Davidson et al. (2011).

2.4. Sampling protocols

Fish were sampled for lengths and weights on a monthly basis and mortalities were removed and recorded daily to assess cumulative survival. During the final fish sampling event of Study 2, notations were made for fish that had any form of curved spine, including kyphosis and lordosis (ventral and dorsal spinal deviations in the axial plane, respectively); scoliosis (spinal deviations in the sagittal plane); or any combination of these observable abnormalities. Skeletal deformities were then summed and divided by the total number of fish sampled per WRAS to determine a percentage of the population affected.

Water samples were collected weekly from the side drain of each tank and tested for a variety of parameters and a series of dissolved metals and elements were analysed when fish were at near-maximum densities and feed loading rates (Davidson et al., 2011). Specific methodologies and laboratory information for all water quality analyses were described in Davidson et al. (2011).

2.5. Rainbow trout swimming speed and behavior observations

Two distinct differences in rainbow trout swimming behavior were observed between treatments during these studies: (1) swimming speed and (2) prevalence of side swimming fish, i.e., fish swimming oriented on their side. Swimming speeds were quantified weekly by timing individual fish passing between marked locations distanced 3 ft apart and then adding the water rotational velocity within 30 cm of the tank wall. Swimming speeds of 15 fish were measured within each tank weekly, including five fish near the top, middle, and bottom of the tanks. Swimming speed measurements for Study 1 began after 7 weeks when it became evident that a distinct difference existed between the high exchange and low exchange treatments. During Study 2, measurements were taken only during the first 9 weeks of the study when water quality was still clear enough to observe fish in the non-ozonated WRAS.

Side swimming behavior was assessed during Study 1 by positioning a video camera directly above the center of each tank. Video footage was collected for the first time approximately 4 months into the study. Five minutes of video were collected for each WRAS. Black and white snap shots of the video were then clipped out at 1 min intervals and side swimming fish, which had a distinct white appearance in the picture, were manually counted. Mean numbers of side swimmers were then calculated and compared between treatments. Side swimming was not quantified during

Table 1
Experimental design overview of water exchange, feed loading rate, hydraulic retention time, and use of ozone for each treatment utilized during Studies 1 and 2.

Water exchange	Number of WRAS	Feed loading (kg feed/m^3 makeup flow/day)	Hydraulic retention time (days)	Ozone
Study 1				
High	3	0.41	0.67	0 of 3
Low	3	4.10	6.70	3 of 3
Study 2				
Very low	3	17–44	25–36	0 of 3
Near-zero	2	71–147	103–180	2 of 2

Note: WRAS 3 was excluded from most analyses, because of the low exchange systems for Study 2, it was the only system that used ozone and also had significantly greater flushing and significantly lower feed loading rates. Therefore, it was considered an outlier.

Study 2, because the water quickly became too turbid to observe this behavior in the non-ozonated WRAS.

2.6. Statistical analysis

Statistical comparisons of swimming speed and number of side swimming fish were made using a Student's t-test. Transformations were applied to abnormally distributed data. All parameters that were sampled during multiple events over time from the same location, such as water quality parameters were analysed using a Hierarchical Mixed Models approach called Restricted Maximum Likelihood (REML), which allows the assignment of "Tank" as a random factor, thus buffering the main treatment effect from potential variation arising from tank effects. A hierarchical approach was recommended by Ling and Cotter (2003), who suggested that the random variation between replicated tanks represents a "nuisance factor" in aquaculture experiments. A probability value (α) of 0.10 was used to determine significance for each statistical test as opposed to the traditional 0.05 due to a relatively low n-value (three WRAS per treatment). Statistical correlation analysis was used to evaluate the strength of relationships between various fish health and welfare metrics and specific water quality parameters. Statistical analyses were carried out using SYSTAT 11 software (2004).

3. Results

3.1. Rainbow trout health and welfare

3.1.1. Swimming speed

Rainbow trout swimming speed generally increased as the WRAS flushing rate decreased and when the system hydraulic retention time was longer. For example, during Study 1, mean swimming speeds in WRAS operated at high exchange were 35.9, 17.5, and 15.9 cm/s; while trout within WRAS operated at low exchange swam at mean speeds of 49.3, 48.1, and 42.6 cm/s (P = 0.056). Statistical comparison indicated that trout within the low exchange WRAS swam at a significantly greater mean speed (1.4 ± 0.1 body lengths/s (bl/s)) than fish cultured within WRAS operated at high exchange (0.7 ± 0.2 bl/s) (P = 0.041) (Fig. 1). Feed loading rate (kg feed/day per m^3 makeup water/day) appeared to be a more correlative metric with rainbow trout swimming speed rather than flushing rate alone. Daily feeding gradually decreased over the course of the study as fish grew larger, thus feed loading decreased and the concentrations of various water quality components were reduced. These changes occurred in unison with the reduction in rainbow trout swimming speed that was evident from the third to sixth month of Study 1 (Fig. 1). Daily observations indicated that trout tended to maintain the described swimming speeds for each condition continuously without rest. During Study 1, fish within the low exchange WRAS were always observed swimming faster than the water rotational velocity, while fish within the high exchange WRAS generally held position in the water column.

During Study 2, rainbow trout swimming speed was generally greater, but not significantly, in WRAS with higher HRT's and feed loading rates. Fish within the very low exchange WRAS had mean swimming speeds of 44.8, 30.6, and 44.1 cm/s, while swimming speeds in the near-zero exchange WRAS were 45.7 and 46.1 cm/s. Rainbow trout stocked during Study 2 were smaller (18 g) than those stocked during Study 2 (151 g), thus swimming speed relative to body length was greater and ranged from 2.1 to 3.4 bl/s.

3.1.2. Rainbow trout swimming behavior

In addition to the swimming speed differences measured between treatments during Study 1, other obvious differences in rainbow trout swimming behavior were observed. Specifically, a statistically greater portion of the population within the low exchange WRAS were observed swimming on their sides in comparison to the high exchange WRAS (P = 0.001) (Figs. 2 and 3). Count data from video snap shots taken 4 months into Study 1 indicated 42 ± 1 side swimming trout in the low exchange WRAS and 10 ± 2 side swimming trout in the high exchange WRAS. Figs. 2 and 3 illustrate the statistically greater number of side swimmers within the low exchange WRAS. Video recordings taken near the end of Study 1, i.e., after 6 months, indicated similar results. At that time, counts of side swimming trout from the low and high exchange WRAS were 26 ± 7 and 10 ± 1 side swimmers, respectively. During Study 2, trout within the near-zero exchange WRAS exhibited additional unusual behaviors including erratic swimming, swimming near the water surface (surface swimming), and periodically swimming at an oblique angle to the surface with their nose out of the water.

3.1.3. Rainbow trout deformities

During Study 1, a difference in the prevalence of rainbow trout deformities was not observed between treatments. However, during Study 2, a higher incidence of skeletal deformities (as pictured in Fig. 4) were observed, particularly in WRAS operated with the

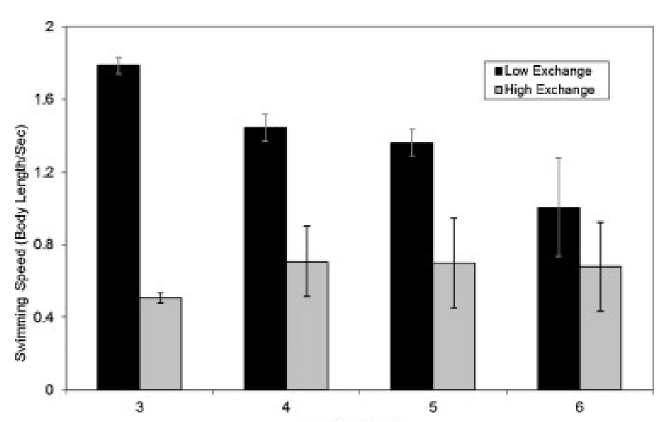

Fig. 1. Mean rainbow trout swimming speeds (±1 standard error) measured from the third to sixth month of Study 1 within WRAS operated with high water exchange vs. low water exchange ozone.

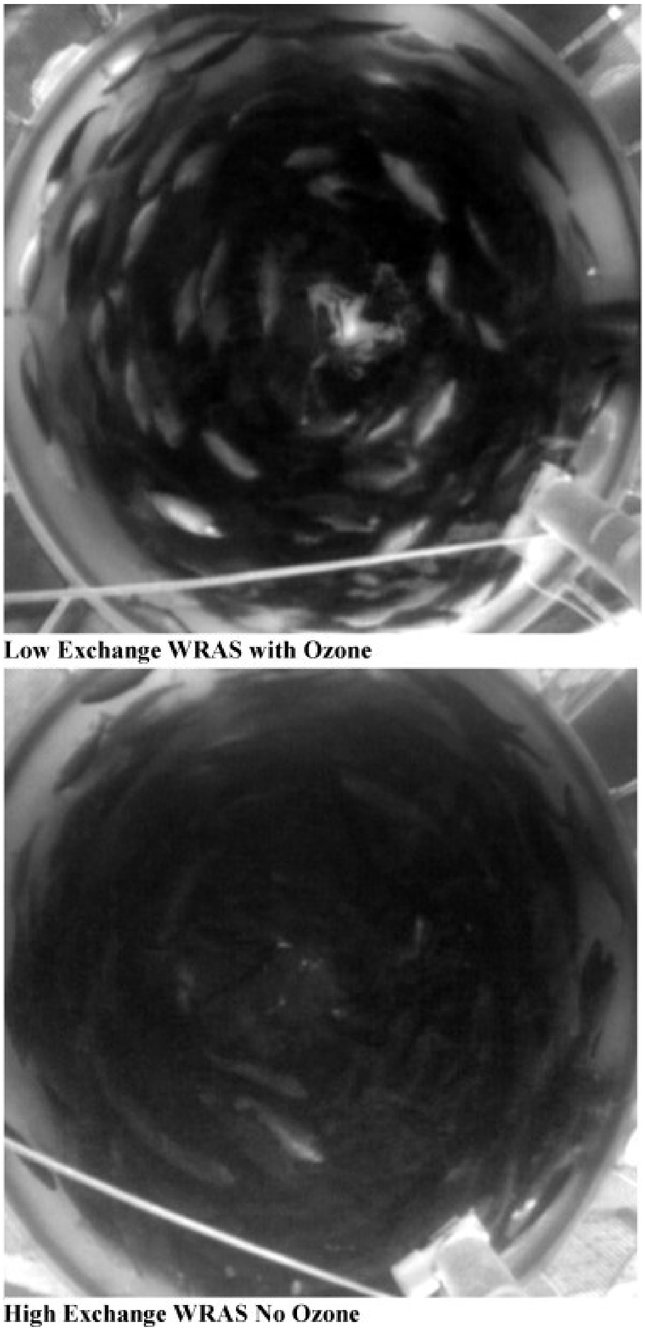

Fig. 2. Video frames of "side swimming" rainbow trout within WRAS operated at low water exchange with ozone and high water exchange without ozone (Study 1).

least flushing and greatest feed loading, i.e. near-zero exchange. For example, the WRAS with the least flushing (HRT = 180 days) had the greatest prevalence of skeletal deformities, 38%, while WRAS with the greatest flushing (HRT = 5 days), had no observable skeletal deformities, 0%. Fish within the near-zero exchange WRAS were also observed as having stiffened musculature during handling.

3.1.4. Decreased survival

During Study 1, rainbow trout survival was excellent for all WRAS and was similar between low exchange WRAS and high exchange WRAS, i.e. 93.3 ± 1.6% and 93.1 ± 0.5%, respectively. Therefore, the flushing and/or feed loading rates did not appear to impact survival during Study 1. During Study 2, WRAS operated at near-zero exchange had substantially greater mortality in

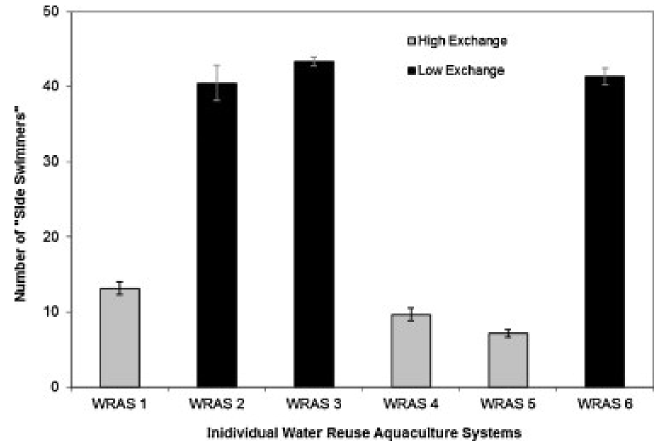

Fig. 3. Number of side swimming rainbow trout (±1 standard error) counted from video frames from individual WRAS during Study 1, comparing WRAS operated at low water exchange with ozone vs. high water exchange without ozone.

comparison to all other WRAS. Mean cumulative survival for the near-zero exchange WRAS (mean HRT ≥103 days and feed loading ≥71 kg feed/day per m^3 makeup water/day) was 85.7 ± 1.9%, while mean survival for the very low exchange WRAS (HRT's of ≤36 days and mean feed loading rates ≤44 kg feed/m^3 makeup water/day) was 94.6 ± 0.4%.

3.2. Water quality concentrations

An extensive suite of water quality parameters were analysed during both studies. Water quality concentrations measured over the duration of each study are presented in Table 2 and dissolved metals concentrations from samples taken during near-maximum feed loading periods are presented in Table 3.

Fig. 4. Examples of skeletal deformities observed in rainbow trout cultured within near-zero exchange WRAS during Study 2.

Table 2
Mean water quality values (±1 standard error) at the tank side drains over the duration of Studies 1 and 2 between systems operated at various water exchange rates. Means for most parameters during Studies 1 and 2 derived from 22 and 17 weekly sampling events, respectively.

	Study 1		Study 2	
Treatment	Low exchange	High exchange	Very low exchange	Near-zero exchange
TAN[*1*2]	0.31 ± 0.02	0.45 ± 0.01	0.92 ± 0.09	0.77 ± 0.05
NH_3	0.003 ± 0.000	0.003 ± 0.000	0.008 ± 0.001	0.005 ± 0.000
NO_2–N	0.11 ± 0.04	0.08 ± 0.00	0.13 ± 0.01	0.13 ± 0.09
NO_3–N[*1*2]	13 ± 0	99 ± 7	171 ± 16	422 ± 13
Alkalinity[*1]	224 ± 3	200 ± 1	216 ± 3	209 ± 3
pH[*1*2]	7.61 ± 0.01	7.47 ± 0.01	7.54 ± 0.03	7.44 ± 0.02
CO_2	10 ± 1	11 ± 0	14 ± 1	16 ± 0
$cBOD_5$[*1*2]	2.5 ± 0.1	3.0 ± 0.2	11.8 ± 2.7	3.7 ± 0.2
TOC	11.2 ± 2.1	17.9 ± 2.8	–	–
DOC	9.0 ± 1.2	16.1 ± 1.6	–	–
True color[*1*2]	12 ± 0	5 ± 1	157 ± 25	5 ± 1
UV transm. (%)[*1*2]	89 ± 0	77 ± 2	30 ± 2	61 ± 0
Phosphorous[*1*2]	0.8 ± 0.0	3.9 ± 1.0	5.2 ± 0.0	9.3 ± 0.8
TSS[*1*2]	3.4 ± 0.1	4.6 ± 0.5	18.9 ± 1.1	3.5 ± 0.6
Heterotrophic bacteria	117 ± 23	114 ± 19	825 ± 407	61 ± 7
Temperature (°C)	12.9 ± 0.0	13.0 ± 0.1	15.7 ± 0.0	15.6 ± 0.1
Conductivity	–	–	2.7×10^3	4.7×10^3
DO[*1*2]	10.4 ± 0.0	10.6 ± 0.0	9.7 ± 0.0	11.1 ± 0.1
ORP[*1*2]	195 ± 8	238 ± 2	156 ± 12	265 ± 6

Note: Mean ORP levels include days when ozone was turned off and are therefore slightly below the ORP ranges described in Section 2.
* Indicates statistically significant between treatments ($P < 0.10$), 1, or 2 following * indicates study 1 or 2.

4. Discussion

4.1. Health and welfare

A variety of unusual swimming behaviors were noted during Studies 1 and 2 that correlated with WRAS water exchange and feed loading rates. The prevalence of each of these behaviors was always greater within WRAS that were operated with less water exchange or greater feed loading, which in turn contained the highest ionic and water quality concentrations.

The authors hypothesize that the increased swimming speeds were a physiological response (similar to a flight response) caused by chronically stressful water quality concentration(s). The observations of increased rainbow trout swimming speed with increasing HRT are important from a fish health and welfare perspective for several reasons: (1) the increased swimming speeds represented a deviation from typical swimming behavior. Given a sufficient rotational velocity, salmonids generally hold position in the water column, as was observed in the high exchange WRAS during Study 1. (2) Increased swimming speeds can result in dramatic increases in oxygen consumption in fish (Brett, 1973; Forsberg, 1994). (3) The mean swimming speeds measured during Study 2 (2.1–3.4 bl/s) and those measured during the third month of Study 1 (1.8 ± 0.0 bl/s) (Fig. 1), exceeded the recommendations of Davison (1997), who provided an overview of literature on the effects of exercise training in fish. Davison (1997) concluded that swimming speeds ≤1.5 bl/s were optimal for growth and feed conversion and suggested that sustained swimming at speeds >1.5 bl/s could have negative impacts on fish. Additionally, Jain et al. (1997) determined that the "fatigue velocity" for rainbow trout was 2.1 ± 0.1 bl/s; therefore, it is possible that rainbow trout were swimming at exhaustive speeds during Study 2. (4) Lastly, excessive swimming activity can cause the accumulation of lactic acid in the blood (lactic acidosis), which can contribute to mortality when fish are severely exercised (Wedemeyer, 1996).

The authors have observed side swimming behavior in a small percentage of rainbow trout previously cultured on-site. The percentage of side swimming trout observed during Study 1 far

Table 3
Mean dissolved metal and nutrient concentrations (mg/L) (±1 standard error) at the tank side drain outlets when WRAS were operated near-maximum feed loading and fish density during Studies 1 and 2. Means for Study 1 derived from one sampling event at near-maximum feed loading. Means for Study 2 derived from two sampling events at near-maximum feed loading.

	Study 1		Study 2	
Treatment/parameter	High exchange	Low exchange	Very low exchange	Near-zero exchange
Barium[*1]	0.055 ± 0.001	0.043 ± 0.001	0.367 ± 0.066	0.228 ± 0.011
Boron	<MDL	<MDL	0.061 ± 0.011	0.079 ± 0.000
Calcium[*1*2]	108 ± 0	104 ± 1	99 ± 2	71 ± 1
Copper[*1*2]	0.014 ± 0.002	0.038 ± 0.004	0.119 ± 0.008	0.050 ± 0.010
Iron[*2]	<MDL	<MDL	0.041 ± 0.013	0.006 ± 0.001
Magnesium[*1*2]	12.1 ± 0.1	14.8 ± 0.4	19.8 ± 0.4	26.2 ± 0.1
Manganese	<MDL	<MDL	0.008 ± 0.004	<MDL
Phosphorous[*1*2]	0.5 ± 0.1	2.7 ± 0.2	7.0 ± 1.6	14.5 ± 0.0
Potassium[*1*2]	5 ± 0	25 ± 3	44 ± 7	112 ± 10
Silicon	48 ± 0	43 ± 2	44 ± 1	41 ± 2
Sodium[*1*2]	5 ± 0	164 ± 20	346 ± 86	753 ± 70
Strontium[*1*2]	0.90 ± 0.00	0.83 ± 0.01	0.89 ± 0.02	0.72 ± 0.00
Sulfur[*1*2]	9.5 ± 0.2	18.4 ± 1.1	26.7 ± 2.0	48.3 ± 1.7
Zinc	0.011 ± 0.003	0.007 ± 0.002	0.128 ± 0.023	0.082 ± 0.000

<MDL = less than minimum detection limit of the test. *Notes:* The following elements were below the minimum detection limit within the culture water for all treatments during both studies: aluminum, arsenic, beryllium, cadmium, chromium, cobalt, lead, mercury, molybdenum, nickel, and selenium.
* Indicates statistically significant between treatments ($P < 0.10$), 2, or 3 following * indicates study 2, or 3.

exceeded that of previously cultured cohorts and therefore was viewed as a potential concern for the health and welfare of the fish. Unfortunately, very little information is available in the literature regarding side swimming behavior in fish. The authors hypothesize that constant increased swimming speeds in the same continuous circular pattern without rest could have caused physiological or morphological changes, such as imbalance in musculature symmetry, skeletal deformities, or a deviation of swim bladder shape and positioning, which may have contributed to the increased side swimming behavior observed in the population. The anatomical and physiological changes associated with side swimmers, however, require further investigation to provide greater understanding of this phenomenon.

Other unusual behaviors were observed during Study 2 in rainbow trout cultured within WRAS with the least flushing and greatest feed loading rates. Specifically, rainbow trout within the near-zero exchange WRAS began to swim erratically several months into the study. Some fish swam near the surface with their bodies at an oblique angle as opposed to fish swimming normally, parallel to the water column. Many of the erratically swimming fish broke the surface of the water with their nose pointed up and exhibited a yawning or gulping action with their mouth. Observation of these unusual swimming behaviors increased as the study progressed and could be defined as severe near the end of the study. The authors hypothesize that the various abnormal swimming behaviors observed during Study 2 could have induced the increase in skeletal deformities. Divanach et al. (1997) concluded that intense posterior muscular activity in sea bass exposed to consistently strong currents induced lordosis. Therefore, it is feasible that rainbow trout swimming at increased speeds always in the same circular direction could have been prone to skeletal deformation during the present studies. Additionally, Kitajima et al. (1994) associated lordotic deformation of the skeleton in hatchery-bred physoclistous fish with an abnormal swimming behavior in which fish swam at an oblique angle to the water surface to compensate for deflated swim bladders. The behavior observed during Kitajima et al.'s study caused a V-shape curvature of the backbone in fish displaying this behavior. During Study 3, rainbow trout were noticed swimming at an oblique angle to the water surface in the near-zero exchange WRAS. Based on Kitajima et al.'s findings, this behavior could have been related to the increase in skeletal deformities observed within these systems, particularly for deformed trout with heads that appeared to curve upward, causing a V-shape of the spine (Fig. 4; fish at top). Skeletal deformities can be a serious economic problem in commercial aquaculture. Deformed fish are often culled from the population or have reduced market value.

Many water quality parameters have been cited as causes, as previously discussed. Although it is apparent that elevated concentrations of various water quality criteria can contribute to skeletal abnormalities, many other parameters have also been implicated. For example, skeletal deformities in cultured salmonids have been attributed to: incubation temperature (Lein et al., 2009), diet and nutrition (Madsen and Dalsgaard, 1999; Power, 2009), genetics (McKay and Gjerde, 1986), and methods used to induce triploidy (Madsen et al., 2000; Sadler et al., 2001; Fjelldal and Hansen, 2010). The fish that were used during the present studies were hatched under the same conditions at the same time, were from the same diploid cohort, and were fed the same diet throughout their life cycle. The skeletal deformities that were observed during Study 2 materialized during the study period, and were therefore at least partially, if not entirely, related to the environmental conditions created during this study.

In addition to the fish health and welfare issues observed, survival also appeared to be related to flushing and feed loading rate during Study 2. Therefore, some aspect(s) of the water quality within the near-zero exchange WRAS likely reached chronic to slightly acute concentrations in order to cause the low level mortality observed.

Each of the aforementioned fish health and welfare metrics appeared to be correlated to feed loading and system flushing rates which suggests that accumulating water quality constituents were related to the observed problems. Therefore, a brief review of the water quality concentrations measured during each of these studies is warranted and provides valuable information and direction for future studies designed to identify accumulating water quality variables that become problematic in low and near-zero exchange WRAS.

4.2. Water quality

Of the water quality parameters measured during Study 1 (Tables 2 and 3), some could systematically be excluded as potential causative agents of the aforementioned health and welfare problems due to: (1) lack of detection during laboratory analyses; (2) concentrations that were significantly lower within WRAS in which health and welfare issues were observed; and (3) concentrations that were not significantly different between WRAS in which health and welfare problems were observed. The remaining water quality parameters that were significantly greater within WRAS in which fish health and welfare issues occurred (i.e., low exchange (Study 1) and near-zero exchange (Study 2)) were further considered as potential causative agents of the observed problems. Water quality parameters are grouped within each of the aforementioned statistical categories in Tables 4 and 5.

The potential toxicity of each water quality concentration that was statistically greater within the low exchange WRAS (Study 1, Table 4) and near-zero exchange WRAS (Study 2, Table 5) were assessed based on toxicity information available in the literature. Davidson et al. (2009, 2011) reviewed recommended upper limits for a variety of metals and water quality parameters as reported in literature (Piper et al., 1982; Meade, 1989; Heinen, 1996; Wedemeyer, 1996; EPA, 1987, 1996, 2002, 2007; Colt, 2006; Boyd, 2009). Of these parameters, nitrate nitrogen, copper, and potassium were categorized as existing at potentially toxic concentrations during Study 1. Statistical correlation analysis indicated that copper, potassium, and nitrate nitrogen correlated well with the number of side swimming fish, as well as fish swimming speed during Study 1. Pearson's correlation coefficient (R) for copper, potassium, and nitrate nitrogen was 0.937, 0.960, and 0.977, respectively, relative to the number of side swimming fish; and 0.916, 0.935, 0.881, respectively, relative to rainbow trout swimming speed.

During Study 2, statistical analysis indicated that copper did not correlate well with swimming speed, deformity, or survival, but indicated a strong correlation of potassium and nitrate nitrogen to each of these metrics. Pearson's correlation coefficient for copper, potassium, and nitrate nitrogen was 0.052, 0.636, and 0.667, respectively, relative to swimming speed; 0.049, 0.609, and 0.762, respectively, relative to deformity; and 0.396, 0.880, and 0.971, respectively, relative to survival.

The authors are fully aware that all water quality parameters that could accumulate within low and near-zero exchange WRAS were not measured during the present studies. Concentrations of other unmeasured parameters could have been related to the fish health and welfare problems observed. For example, pheromones secreted by the fish could accumulate within WRAS and could cause an alarm reaction or other impacts to fish behavior (Solomon, 1977; Colt, 2006). In addition, endocrine disrupting chemicals including pesticides, natural and synthetic hormones, and PCB's could accumulate within WRAS if present within the makeup water supply and could cause adverse effects to fish (Damstra et al., 2002; Colt, 2006). Furthermore, plasticizers and/or trace contaminants from

Table 4
Systematic grouping of measured water quality parameters relative to statistical analysis, used to facilitate identification of water quality parameters that could have been related to the fish health and welfare problems observed during Study 1.

<Detection within low exchange	Significantly < within low exchange	No significant difference between high and low exchange	Significantly > within low exchange
Aluminum	Barium	Unionized ammonia	Copper
Arsenic	Calcium	Nitrite nitrogen	Magnesium
Beryllium	Strontium	Carbon dioxide	Phosphorous
Boron	True color	Total organic carbon	Potassium
Cadmium	UV transmittance	Dissolved organic carbon	Sodium
Chromium		Heterotrophic bacteria	Sulfur
Cobalt		Temperature	Total ammonia nitrogen
Iron			Nitrate nitrogen
Lead			Alkalinity
Manganese			pH
Mercury			Biochemical oxygen demand
Molybdenum			Total suspended solids
Nickel			Dissolved oxygen
Selenium			ORP

PVC or fiberglass could leach from system components, accumulate within WRAS, and potentially cause negative impacts to cultured species (Carmignai and Bennett, 1976; Zitko et al., 1985; Colt, 2006). In addition, interacting or combined effects of various water quality parameters (measured and/or unmeasured), as well as the overall conductivity or ionic concentration of the culture environment could have been responsible for the observed fish health and welfare problems.

The following discussion is meant to focus on the few measured parameters that were separated as being potentially related to the described fish health and welfare issues, which will be beneficial to future research regarding the toxicity of specific water quality parameters within low and near-zero exchange WRAS.

4.2.1. Ozone

Aside from water exchange rate, a distinct difference between treatments during Studies 1 and 2 was the use of ozone; therefore a brief toxicity review was warranted. During each study, ozone was generally used within WRAS that were operated at lower water exchange rates, i.e., WRAS in which the majority of abnormal swimming behaviors and other negative health and welfare effects were observed. Therefore, ozone toxicity was stringently evaluated. Bullock et al. (1997) suggested that an ORP level of 300 mV was safe for rainbow trout, and Summerfelt et al. (2009) reported that mean dissolved ozone concentrations were 0 ppb at a mean ORP $\leq$340 mV. To ensure that ozone did not remain in the culture water at toxic concentrations during the present studies, ozone residual was monitored and controlled using ORP. Mean ORP levels recorded over the duration of Studies 1 and 2 within WRAS operated with ozone were 238 ± 2 and 265 ± 6 mg/L, respectively (Table 2), thus ORP was maintained well below the threshold at which ozone residual becomes problematic for fish (Bullock et al., 1997; Summerfelt et al., 2009). Study 2 results further vindicated ozone residual as a cause for the negative impacts on fish health and welfare, because WRAS 3, which was operated with ozone, did not exhibit the previously described abnormal rainbow trout swimming behaviors, skeletal deformities, or decreased survival. In addition, previous on-site studies have been conducted using a similar ozone dose within a commercial scale WRAS culturing salmonids (Summerfelt et al., 2009), without any of the consequences to fish that are described in this paper. Thus, ozone residual was not suspected as a possible cause of the observed fish health and welfare problems.

4.2.2. Copper

Davidson et al. (2009) provided an overview of literature regarding the toxicity of copper to salmonids. In summary, the chronic-acute limits for dissolved copper are 0.022–0.037 mg/L at a corresponding water hardness of 300 mg/L as $CaCO_3$ (Alabaster and Lloyd, 1982; EPA, 2002). Water hardness measured during the present studies ranged from 290 to 312 mg/L as $CaCO_3$. In addition to hardness, alkalinity, pH, temperature, dissolved organic carbon (DOC), and TSS (Spear and Pierce, 1979; Alabaster and Lloyd, 1982; Sprague, 1985; U.S. EPA, 2002, 2007), can interact to alter copper toxicity. Updated U.S. EPA (2007) guidelines for copper toxicity which account for hardness, as well as DOC indicate that the chronic-acute copper toxicity limits could have been at least four times greater ($\geq$0.088–0.148 mg/L) than earlier EPA models predicted (0.022–0.037 mg/L) at the same alkalinity (200 mg/L). Based on this toxicity review, rainbow trout in the low exchange WRAS

Table 5
Systematic grouping of water quality parameters based on statistical analysis, used to facilitate separation of water quality parameters that could have been related to the fish health and welfare problems observed during Study 2.

<Detection within near-zero exchange	Significantly < within near-zero exchange	No significant difference between very low and near-zero exchange	Parameters significantly > within near-zero exchange WRAS
Aluminum	Calcium	Barium	Magnesium
Arsenic	Copper	Boron	Phosphorous
Beryllium	Iron	Silicon	Potassium
Cadmium	Strontium	Zinc	Sodium
Chromium	Total ammonia nitrogen	Nitrite nitrogen	Sulfur
Cobalt	Unionized ammonia	Alkalinity	Nitrate nitrogen
Lead	pH	Carbon dioxide	UV transmittance
Mercury	Biochemical oxygen demand	Heterotrophic bacteria	Conductivity
Manganese	True color	Temperature	Dissolved oxygen
Molybdenum	Total suspended solids		ORP
Nickel			
Selenium			

during Study 1 and in all WRAS during Study 2 would have been negatively impacted by the measured dissolved copper concentrations (0.038–0.119 mg/L) (Table 3) in the absence of other buffering water quality parameters.

4.2.3. Potassium

Potassium accumulated with increasing feed loading rate and HRT (Davidson et al., 2011); thus, mean dissolved potassium levels during Study 1 were approximately five times greater (25 ± 3 mg/L) within the low exchange WRAS (Table 3) in which the abnormal swimming behaviors were observed as compared to the high exchange WRAS (5 ± 0 mg/L). During Study 2, potassium concentrations also accumulated relative to increasing feed loading rate and HRT (Table 3). Dissolved potassium concentrations in the very low exchange and near-zero exchange WRAS were 44 ± 7 and 112 ± 10 mg/L, respectively (Table 3).

Scientific literature typically discusses potassium toxicity relative to compounds such as potassium permanganate or potassium cyanide; therefore, little information is available regarding the toxicity of dissolved potassium alone. One study which evaluated the acute toxicity of potassium permanganate in African catfish *Clarius gariepinus* fingerlings, noted symptoms such as erratic swimming and gulping for air, which seem similar to observations during the present studies (Kori-Siakpere, 2008). Buhse (1974) reported that potassium >200 mg/L was toxic to fish in freshwater environments. Bell (1990) reported that 50 mg/L potassium could be toxic to fish, especially in soft water. Additionally, Heinen (1996) referenced literature that suggested that ≥10 mg/L potassium is acceptable for culture water with hardness >100 mg/L. Additionally, potassium levels of 100–130 mg/L were suspected as the cause for gill problems in rainbow trout (>400 g) in an aquaponics facility that supplemented potassium (personal communication, Marc Laberge, Cultures Aquaponiques Inc., Quebec, CA). With such a wide range of recommendations, it is unclear whether the potassium concentrations measured during the present studies were harmful to rainbow trout, thus further evaluation is needed.

4.2.4. Nitrate nitrogen

Several important publications have stated that NO_3–N is generally nontoxic to fish at concentrations that would be expected under typical culture conditions (Wedemeyer, 1996; Colt and Tomasso, 2001; Timmons and Ebeling, 2007; Colt, 2006). However, few specific studies have been conducted to evaluate the toxicity of NO_3–N to salmonids. Camargo et al. (2005) provided an overview of nitrate toxicity studies conducted with freshwater fish including salmonids. Several of these studies indicated that NO_3–N can be chronically toxic to salmonid eggs and larvae at concentrations <200 mg/L with sublethal effects occurring at <25 mg/L (Kincheloe et al., 1979; McGurk et al., 2006). However, establishment of acute, chronic, and sublethal NO_3–N levels would certainly depend upon life stage (Camargo et al., 2005). Only Westin (1974) evaluated the effects of NO_3–N to fingerling-sized rainbow trout (Camargo et al., 2005). Westin (1974) reported a 96-h LC_{50} of 1364 mg NO_3–N/L and a 7-day LC_{50} of 1068 mg NO_3–N/L for rainbow trout fingerlings. Despite the relatively high NO_3–N levels reported for acute toxicity, Westin (1974) recommended a maximum allowable concentration of approximately 57 mg NO_3–N/L for chronic exposure and only 5.7 mg NO_3–N/L for optimal health and growth of salmonids. During Westin's study, rainbow trout were reported to swim near the surface of the tank exhibiting a yawning or gulping action, and some broke the surface with their nose as if trying to escape. Interestingly, many of the rainbow trout swimming behaviors reported by Westin (1974) due to toxic nitrate nitrogen were similar to those reported during the present studies. In addition, unusual swimming behavior similar to that observed during the present studies including side swimming behavior, as well as stiffened or contracted musculature, have also been observed in seabream cultured in a zero-discharge WRAS when NO_3–N concentrations were 200–300 mg/L (personal communication, Jaap Van Rijn, Hebrew University of Jerusalem, Israel). Several other studies have also concluded that NO_3–N could be a parameter of concern for various species cultured in WRAS that are operated with low water exchange rates, including Martins et al. (2009a) – common carp; Hamlin (2006) – Siberian sturgeon *Acipenser baeri*; and Hrubec (1996) – hybrid striped bass *M. saxatilis* × *M. chrysops*. Therefore, more research is certainly needed to evaluate the chronic NO_3–N toxicity threshold for salmonids that are cultured in WRAS. Such research would enable the establishment of more concrete design limits for NO_3–N within low exchange WRAS used for salmonid culture.

5. Conclusion

The results of the present studies provide strong evidence that some aspect of the water quality environment within the low (HRT = 6.7 days; feed loading rate = 4.1 kg feed/m^3 daily makeup flow) and near-zero exchange (feed loading rate ≥71 kg feed/m^3 makeup flow/day; >103 days HRT's) WRAS caused negative impacts to rainbow trout health and welfare. Some of these impacts were subtle and are best described as chronic, such as increased swimming speeds and side swimming behavior. However, in WRAS with near-zero exchange rates, increased deformities and decreased survival occurred. Of the measured parameters, accumulating dissolved potassium and nitrate nitrogen were separated as possible causes of the observed fish health and welfare problems and should be further evaluated.

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